...es and Polychlorinated Biphenyl Residues in Human Breast Lipids and Their Relation to Breast Cancer

...JR.
...Ophthalmology
...chigan
...higan
..., JR.
...Pathology and Surgery
...al
...cticut
...T
...LD
...onmental and Occupational Medicine
...Community Medicine
...ool of Medicine
...York

...Pathology and Surgery
...al
...cticut

ABSTRACT. The etiology of human breast cancer is unknown; accepted risk factors, e.g., menstrual, reproductive, and family histories, are implicated in less than half of all cases. Various halogenated hydrocarbons—acting as either co-carcinogens or promoting agents—which are derived from the environment and are concentrated in human fatty stores, may also play a role in breast cancer risk. A pilot study was undertaken to measure and compare levels of chemical residues in mammary adipose tissue from women with malignant and nonmalignant breast disease. Elevated levels of polychlorinated biphenyls, bis (4-chlorophenyl)-1,1 dichloroethene, and bis(4-chlorophenyl)-1,1,1 trichloroethane were found in fat samples from women with cancer, compared with those who had benign breast disease. These results, although preliminary, suggest a role for environmentally derived suspect carcinogens in the genesis of mammary carcinoma.

...ENVIRONMENTAL CONTAMINA-...d with several pesticide residues of ...ydrocarbon family, including dichlo-...ethane (DDT), dichlorodiphenyldi-...(DDE), polychlorinated biphenyls ...inated biphenyls (PBBs), benzene ...C), hexachlorobenzene (HCB), diel-...e. These highly persistent, lipophilic, ...emicals have been detected worldwide in wildlife and in humans.[1] These chemicals cause cancer in animals, but evidence for human carcinogenicity is scant. It has also been reported that these compounds possess estrogenic activity,[2] suppress immune function,[3] and induce hepatic microsomal enzymes.[4] These observations, and early studies of breast tumor tissue,[5] suggest that these compounds may increase the risk for some cancers, including mammary carcinoma. Another study found no difference in DDE

and PCBs between cases (n = 14) and controls (n = 21), but the numbers were not large, and the findings deserve corroboration.[6]

We undertook a pilot study to determine whether levels of several chemical residues, including DDT and PCBs, were higher in mammary adipose tissue from women who had malignant breast disease, compared with mammary adipose tissue from women who had benign breast disease.

Materials and methods

Patient population. The study population was derived from a series of 50 Caucasian females seen for initial surgical evaluation of a palpable breast mass or mammographic abnormality at Hartford Hospital (Hartford, Connecticut) from May through September 1987. After frozen section consultation was completed, 0.5 g of grossly nondiagnostic fat was separated from the biopsy or mastectomy specimen, coded, and stored at −70 °C in prewashed (detergent, water, acetone, hexane) vials (American Scientific Products) until forwarded for chemical analysis. The samples were collected consecutively by one pathologist in a nonbiased fashion. The only selection criterion was the presence of gross fat in an amount considered adequate for chemical analysis. Informed consent for tissue examination by the laboratory was obtained in each case. Routine informed consent was adequate for the purpose of this study because the chemical analyses were performed on fat destined to be discarded without further processing.

Twenty-three fat samples were obtained from women who had mammary carcinoma, and the remaining 27 samples were from women who had only benign disease. Fat samples from 40 patients in this initial group (20 benign controls and 20 malignant cases) were chosen for chemical analysis; therefore, the ages for the two groups were similar. Patient height, weight, and smoking histories were obtained from the medical records or from brief telephone interviews. Other data, such as specific dietary histories, were unavailable.

Histopathology. Histologic sections of all breast lesions were prepared from formalin-fixed, paraffin-embedded tissue, stained with hematoxylin-eosin, and examined by one of the authors (AR). Benign disease was classified according to the 1985 Pathologists' Consensus Statement.[7] Carcinomas were classified as in situ and/or invasive ductal or lobular, and all patients were staged according to the cancer criteria of the American Joint Committee.[8]

Chemical analysis. Standard techniques were used to analyze 0.5-g coded samples for hexachlorobenzene; hexachlorocyclohexane (lindane); three chlordane residues (heptachloroepoxide, oxychlordane, trans-nonachlor); DDT residues (bis[4-chlorophenyl]-1,1 dichloroethene [p,p'-DDE], bis[4-chlorophenyl]-1,1,1 trichloroethane [p,p'-DDT]); and PCBs. The method was modified from that reported[9]: 1:1 dichloromethane-cyclohexane was used for automated gel permeation chromatographic lipid removal, and a Florisil column was used to enrich PCBs and pesticides in two fractions. PCBs were calculated as Aroclor 1260 (peaks with retention time greater than that for p,p'-DDE) by the method of Webb and McCall.[10] Concentrations were reported on the basis of both lipid weight (uncorrected) and wet weight, corrected for recovery of an internal standard (aldrin), because variance introduced by the lipid weighing step and by the clean-up steps was significantly reduced by this calculation (F test for among-/within-run variance) for all chemical residues except HCB. Results for 14 quality control samples (i.e., chicken fat fortified with pesticides and Aroclor 1260-PCBs) were, based on lipid weight, 74 ± 22% for HCB and 99–109 ± 5–19% for other residues; and on the basis of wet weight, corrected for aldrin recovery, 59 ± 18% for HCB and 80–88 ± 12–20% for other residues. Lipid constituted 74 ± 5% of the wet weight for the 40 human samples.

In addition to the t tests, comparisons were made for which nonparametric techniques (Wilcoxon rank-sum) were used, but the results were very similar. The medians and geometric means were similar to the arithmetic means. Calculations and statistical analyses were performed with the Statistical Analysis System (SAS Institute, Cary, NC) at the City University of New York Computer Center.

Results

Patient population. The mean ages were similar for the two groups (63 y for cases, 59 y for controls), but age distributions were different; the standard deviations, medians, and ranges for the cases and controls were 13, 64, 36–86 and 8, 58, and 45–76, respectively. More female controls had a history of current or past smoking (15 of 20) than did cases (6 of 20). Average heights and weights, including Quetelet's index (w/h²), were almost identical for the two groups, i.e., average height and weight for cases (n = 18) were 161 ± 5 cm and 68 ± 14 kg, respectively, and 162 ± 6 cm and 71 ± 15 kg, respectively, for controls (n = 18). (Data were not available for four persons.)

Histopathology. The majority of controls showed nonproliferative fibrocystic changes, which were sometime macrocysts or combined with small fibroadenomata. Other histological diagnoses included physiologic changes, proliferative fibrocystic changes without atypia (including one solitary papilloma), and solitary broadenoma. There were no examples of atypical hyperplasia in this series. Sixteen of 20 (80%) cases had ductal carcinoma with an invasive component, 2 of 20 (10%) had invasive lobular carcinoma, and 2 (10%) had only in situ (ductal) disease. Clinicopathologic stages were Tis-2/20, I-7/20, II-8/20, and III-3/20. One four of the cases had a history of contralateral carcinoma. One patient who had invasive ductal carcinoma also had lobular carcinoma in situ in the same breast, and one of 20 patients had 2 discrete breast tumors.

Chemical analysis. Mean concentrations of PCBs and p,p'-DDE were 50–60% higher in tissues of women who had breast cancer (wet weight basis, Table 1). Levels of p,p'-DDT were also elevated among cases. All differences were statistically significant, regardless

Table 1.—Pesticide and PCB Concentrations in Mammary Adipose Tissue (ng/g) from Cases with Breast Cancer (n = 20) and from Controls with Benign Disease (n = 20)

Pesticides or PCB concentrations	Cases		Controls		p*
	$\bar{X} \pm SD$	Range	$\bar{X} \pm SD$	Range	
Wet weight basis, corrected for recovery (ng/g)					
HCB	23 ± 8	13– 42	20 ± 10	12– 54	.32
HX + OC	116 ± 50	41– 203	97 ± 49	25– 249	.22
TN	87 ± 37	24– 182	96 ± 80	236– 394	.65†
DDE	1 877 ± 1 283	337–4 982	1 174 ± 630	237–2 246	.04†
DDT	179 ± 135	60– 686	14 ± 49	44– 248	.05†
PCBs	1 669 ± 894	733–4 674	1 105 ± 424	592–2 609	.02†
Lipid basis, uncorrected (ng/g)					
HCB	28 ± 11	16– 61	26 ± 11	14– 60	.54
HX + OC	136 ± 52	66– 243	121 ± 53	33– 278	.36
TN	103 ± 43	38– 197	118 ± 87	53– 439	.49†
DDE	2 200 ± 1 470	425–6 398	1 487 ± 842	308–3 674	.07†
DDT	216 ± 174	72– 881	148 ± 75	42– 405	.12†
PCBs	1 965 ± 927	827–4 562	1 395 ± 468	823–2 875	.02†

Notes: HCB = hexachlorobenzene, HX + OC = heptachlorepoxide and oxychlordane (the sum), TN = trans-nonachlor, p,p'-DDE = bis(4-chlorophenyl)-1,1-dichloroethene, p,p'-DDT = bis(4-chlorophenyl)-1,1,1-trichloroethane, and PCBs = polychlorinated biphenyls.
*Two-tailed probability for t.
†For t with unequal variances.

whether parametric or nonparametric (Wilcoxon rank-sum) methods were used. Other pesticides were not significantly different in cases and controls. There were differences in age distribution between cases and controls; therefore, logistic regression models were used to confirm the relationship between levels of PCBs, DDE, and DDT and breast cancer status with respect to potential confounding factors (age, Quetelet's index, smoking status). Even though smoking is not accepted as a significant risk factor for breast cancer, it was included because of the marked difference between cases and controls in our study. In these models, PCBs remained significant, DDE remained significant when smoking was not in the model (with smoking, p = .068), and DDT was no longer related to cancer status. Because smoking is not considered a major risk factor for breast cancer, we conclude that the logistic regression models support the simple statistical tests that show differences between cancer and noncancer tissue levels of DDE and PCBs. Logistic regression coefficients are shown in Table 2. The coefficients for DDE and PCBs are approximately 0.001, which suggests that a 10-ppb increase in tissue level corresponds to a 1% increase in risk of breast cancer. However, because other important risk factors for breast cancer were not studied, there are uncertainties in interpretation of these findings, which require verification in a larger study designed to control for other known risk factors.

Discussion

Breast cancer is the most prevalent cancer among American women.[11] However, the identified risk factors account for considerably less than half of breast cancer in the United States. Breast cancer risk has been correlated with reproductive factors, e.g., early age at menarche and late onset of menopause, which suggests that hormonal factors may be involved. Genetic markers and dietary contributions have been implicated in some studies. Variations in rates of breast cancer internationally are not readily explained by these factors,[12] and, therefore, other environmental factors, such as exposure to carcinogenic chemicals, may be part of the explanation. Differences in such exposures might account for disparate rates of breast cancer, e.g., between racial groups and between countries with similar dietary profiles.

The difference in our findings from those of Unger et al.[6] may result from chance or from differences in the study groups, e.g., nationality or other confounding factors. The finding of higher tissue levels among cancer cases may also signify a redistribution of chemicals to the breast during the disease process. These caveats, and the preliminary (or pilot) nature of our findings, necessitate further investigation of the question of environmental chemicals and breast cancer with better age matching and attention to other factors known to contribute to breast cancer, such as parity, age, and menopausal status. Because these chemicals are probably promotors or inducers, rather than direct carcinogens, their involvement, if any, in human cancer may be part of a complex array of genotoxic and epigenetic effects.

In light of increasing rates of breast cancer[13] and our limited knowledge of the causes, investigation of carcinogenic environmental chemical exposures is a

Table 2.—Logistic Regression Coefficients for Breast Cancer in Cases and Controls

Residue	Intercept	Age	Age 2	Residue	Smoking
DDE	29.052*	−1.058*	0.00896*	0.00085†	—
	34.967*	−1.162*	0.00927*	0.00122*	−2.662*
PCB	28.632*	−1.059*	0.00864*	0.0022*	—
	31.251*	−1.088*	0.00859*	0.00234*	−1.658†

Notes: Age is in years; residue is in parts per billion; and smoking ever = 1, never = 0. Quetelet's index did not achieve statistical significance in these models. The standardized coefficients were approximately 0.5.
*$p < .05$.
†$p < .07$.

promising avenue to explore in our efforts to further elucidate the origins of this disease.

* * * * * * * * * * *

Supported in part by National Institute of Environmental Health Sciences grant *ES00928. The authors thank Dr. Robert Rippey for* statistical consultation and Ms. Marilyn Rivera for technical assistance.

Submitted for publication November 20, 1990; revised; accepted for publication June 26, 1991.

Requests for reprints should be sent to: Dr. Frank Falck, Jr., Department of Ophthalmology, University of Michigan, 1000 Wall Street, Ann Arbor, MI 48105.

* * * * * * * * * * *

References

1. Murphy R, Harvey C. Residues and metabolites of selected persistent halogenated hydrocarbons in blood specimens from a general population survey. Environ Health Perspect 60:115–20.
2. Mason RR, Schulte GJ. Interaction of *o,p'*-DDT with the estrogen-binding protein (EBP) of DMBA-induced rat mammary tumors. Res Commn Chem Pathol Pharmacol 1981; 33:119–28.
3. Exon JH, Kerkvliet NI, Talcott PA. Immunotoxicity of carcinogenic pesticides and related chemicals. J Environ Sci Health. Part C. Environ Carcin Rev 1987; C5:73–120.
4. Parkinson A, Robertson L, Safe L, Safe S. Polychlorinated biphenyls as inducers of hepatic microsomal enzymes: structure activity rules. Chem Biol Inter 1980; 30:271–85.
5. Wasserman M, Nogueira DP, Tomatis L, Mirra AP, Shibata H Arie G, Cucos S, Wasserman D. Organochlorine compounds i neoplastic and adjacent apparently normal breast tissue Bull E viron Contam Toxicol 1976; 15:478–84.
6. Unger M, Kiaer H, Blichert-Toft M, Olsen J, Clausen Organochlorine compounds *in human breast fat from decease* without breast cancer and in a biopsy material from new diagnosed patients undergoing breast surgery. Environ Res 198 34:24–28.
7. Hutter RVP. Is fibrocystic disease of the breast precancerou Arch Pathol Lab Med 1986; 110:171.
8. Beahrs OH, Myers MH, Eds. Manual for staging cancer, 2nd e America Joint Committee on Cancer. Philadelphia, PA: JB Lippi cott, 1983; 127–34.
9. Wolff MS, Fischbein A, Thornton J, Rice C, Lilis R, Selikoff Body burden of polychlorinated *biphenyls among perso* employed in capacitor manufacturing. Int Arch Occup Envir Health 1982; 49:199–208.
10. Webb RG, McCall AC. Quantitative PCB standards for electr capture gas chromatography. J Chromat Sci 1973; 11:366–73
11. Kelsey JL, Berkowitz GS. Breast cancer epidemiology Can R 1988; 48:5615–23.
12. Willett W. The search for the causes of breast and colon cance Nature 1989; 338:389–94.
13. Glass AG, Hoover RN. Rising incidence of breast cancer re tionship to state and receptor status. *J Natl Cancer Inst* 1990; 8 693–96.